

Finding **syntrophic relationships** in
microbial communities by **statistical correlation of**
abundance in diverse environments

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Surrounding evidence for **sulfur cycling syntrophy** at Nakabusa hot springs

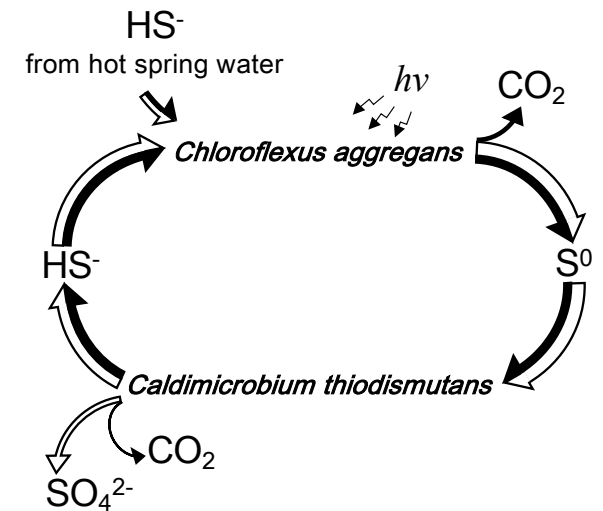
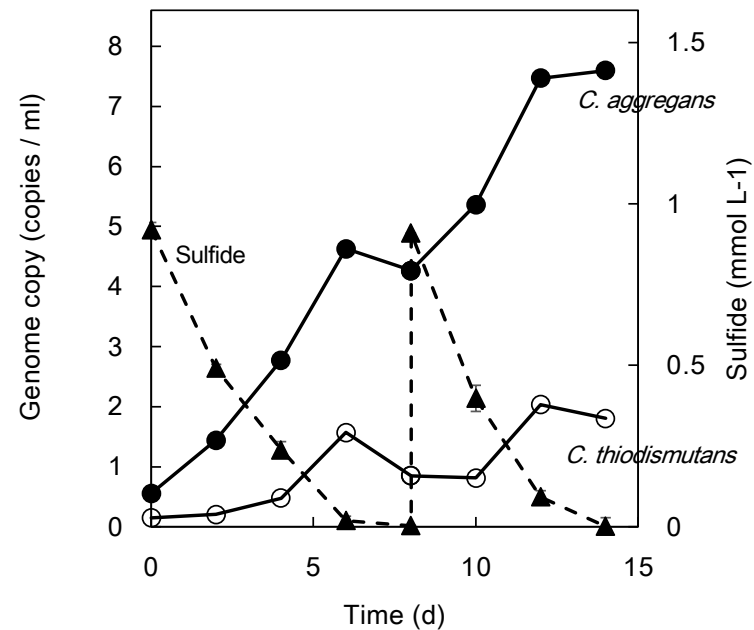
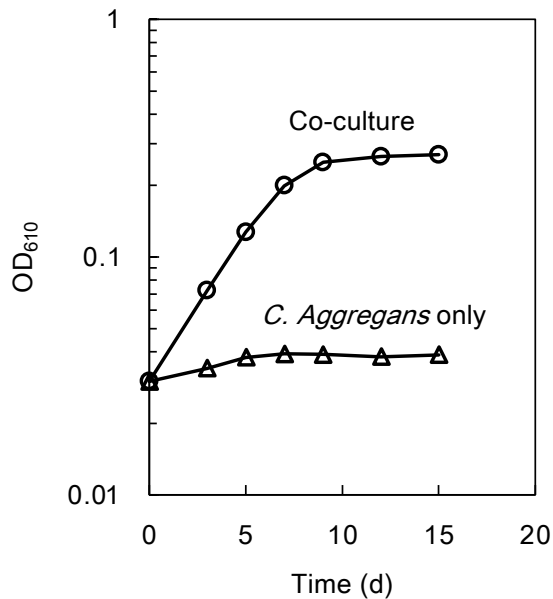
1. An anoxygenic photosynthetic bacterium, *Chloroflexus aggregans*, often **dominates** as a primary producer in Nakabusa hot springs.
2. The major **electron donor** in Nakabusa hot springs is **sulfide** (HS⁻).
3. The **in-vitro culture** of *C. aggregans* **does not grow** well with sulfide*.
4. When **S₀ disproportionating bacterium**, *Caldimicrobium thiodismutans*, was co-cultured, *C. aggregans* **grows** well, suggesting the **syntrophic relationship** of both species*.

*S. Kawai, N. Kamiya, K. Matsuura and S. Haruta (2019) Front Microbiol. 10: 1150

Symbiotic growth of a thermophilic sulfide-oxidizing photoautotroph and an elemental sulfur-disproportionating chemolithoautotroph and cooperative dissimilatory oxidation of sulfide to sulfate

Surrounding evidence for **sulfur cycling syntrophy** at Nakabusa hot springs

In vitro previous study



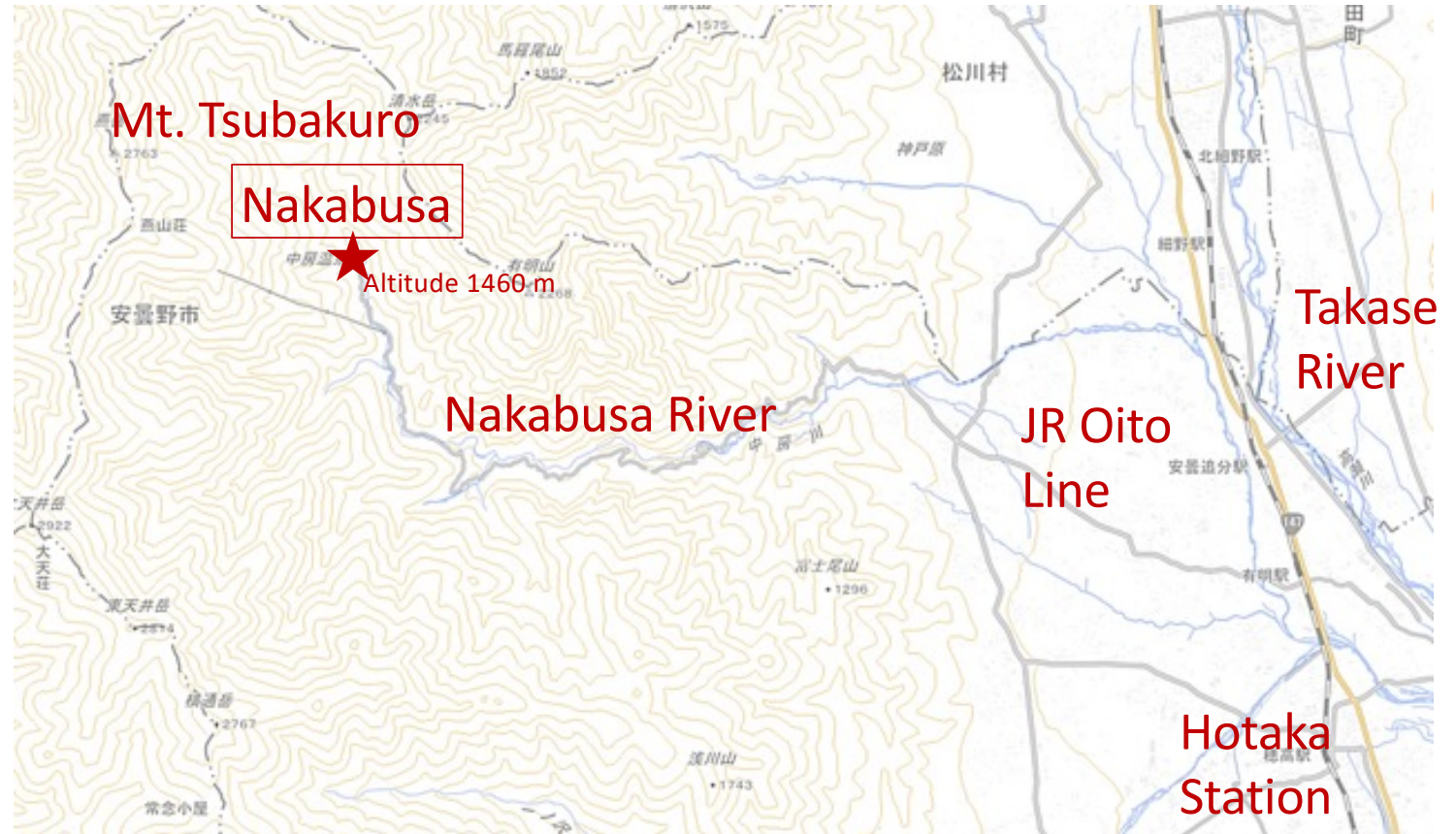
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Symbiotic growth of a thermophilic sulfide-oxidizing photoautotroph and an elemental sulfur-disproportionating chemolithoautotroph and cooperative dissimilatory oxidation of sulfide to sulfate

Is there a **natural sulfur syntrophy** at Nakabusa hot springs???

1. Examine the **syntrophic relationship** between *C. aggregans* and *C. thiodismutans* in **natural** microbial communities.
2. Evaluate the **statistical abundance-correlation** analysis for symbiotic growth of microbes in natural communities.
3. Find microbes that are **symbiotic and counter-symbiotic** with *C. aggregans*.
4. Explore the possibility of inferring the **lifestyle of uncultured microbes** by the statistical abundance-correlation analysis.

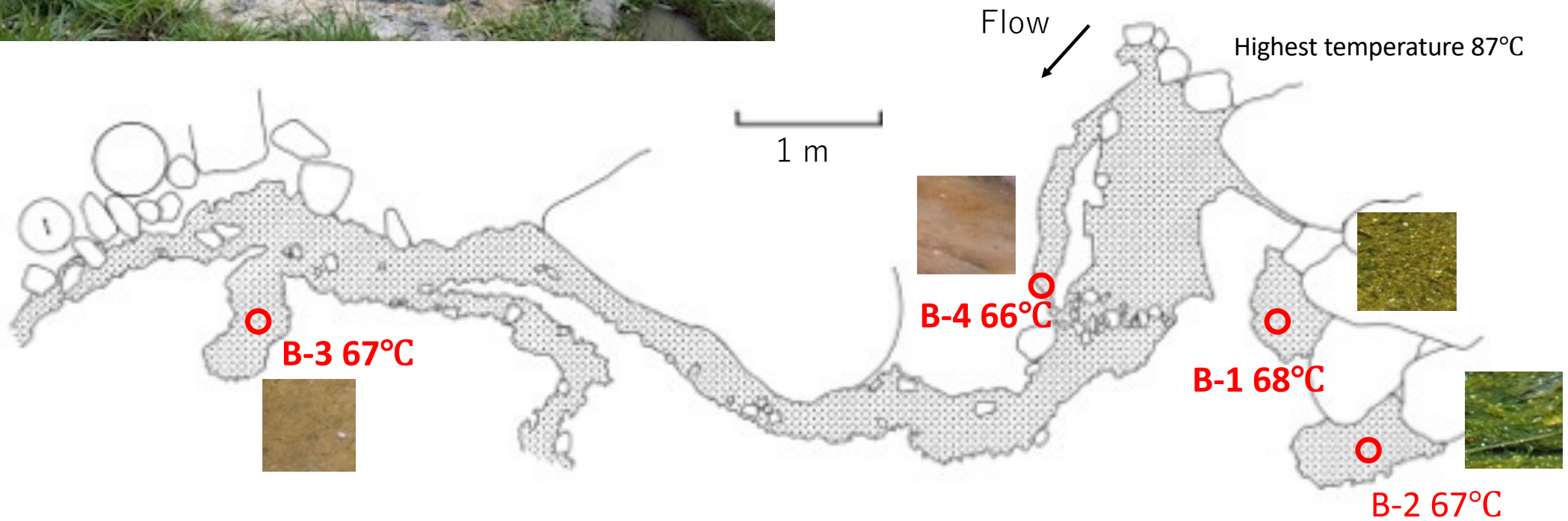
Nakabusa Hot Springs in Nagano



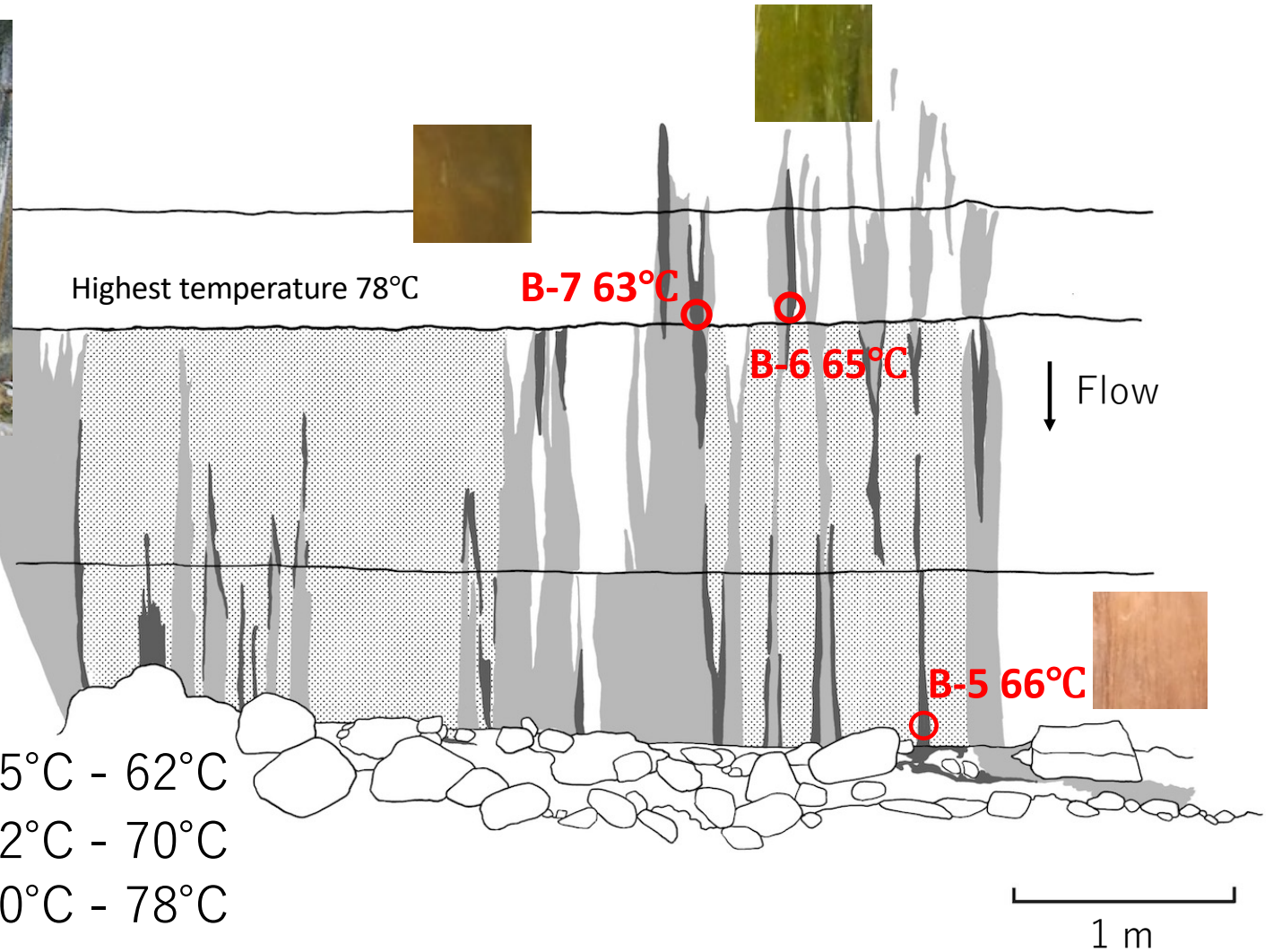
More than 30 springs
Temperature 60°C~95°C
Outflow 1500 L/min

pH 8.0~9.5
Sulfide 200~400 μmol/L
Sulfate 150~300 μmol/L
Carbonate 1.5~2.5 mmol/L

Research site 1: **Kassen spring**: Flow on the sandy ground










Research site 2: **Kojiki spring**: Flow on the concrete wall



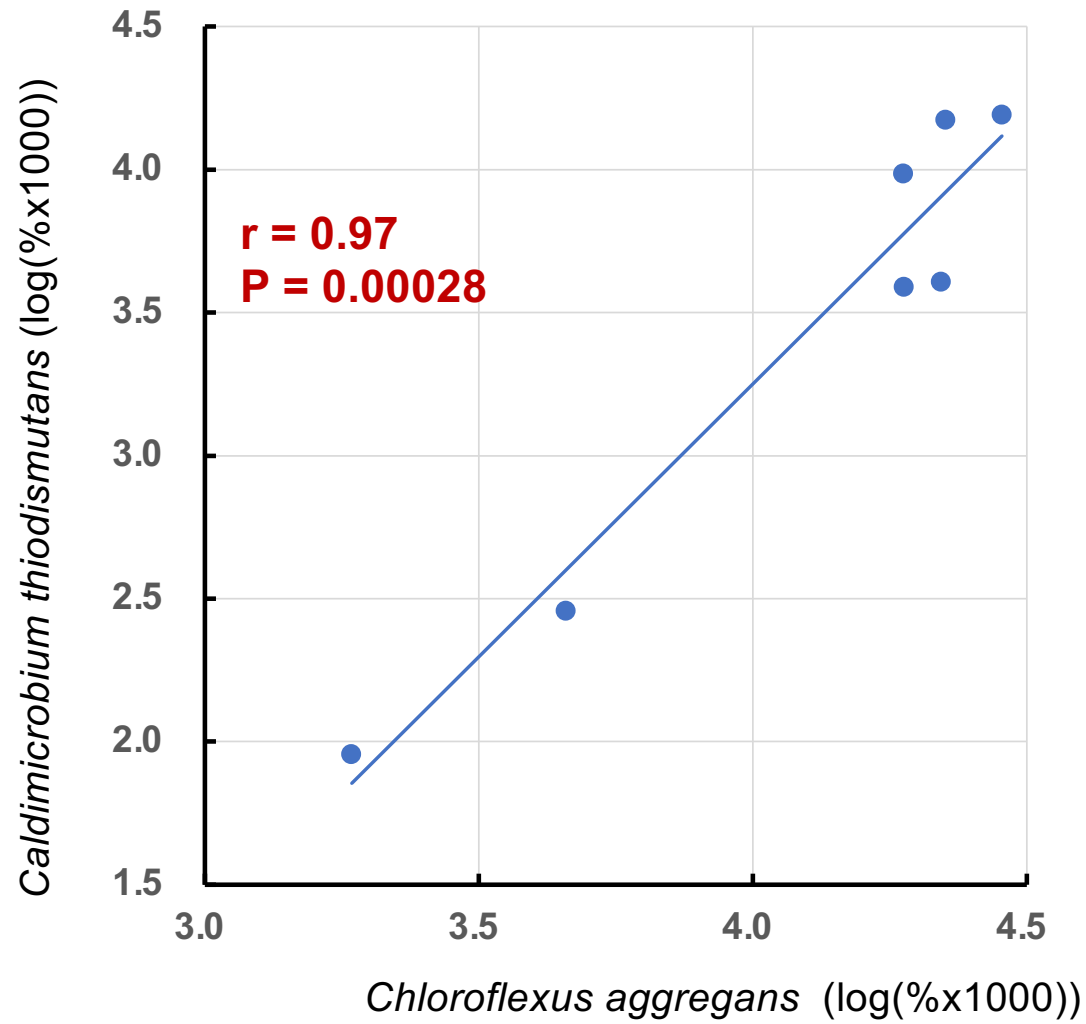
The amplicon of 16S rRNA V4 region was sequenced in 7 communities.

High abundance of *Chloroflexus* and *Caldimicrobium* in diverse environments at 60-70°C in Nakabusa hot springs




Log (% x 1000)

Sample number			B1	B2	B3	B4	B5	B6	B7
Temperature (°C)			68	67	67	66	66	65	63
Sulfide concentration (μmol/L)			202	109	14	22	2	146	58
O ₂ concentration (μmol/L)			42	33	74	130	134	84	116
									
nearest sp.	identity	ratio							
<i>Chloroflexus aggregans</i>	100.00	16.74%	4.28	4.45	4.34	3.27	3.66	4.35	4.28
<i>Sulfurihydrogenibium azorense</i>	100.00	9.26%	3.92	2.76	3.64	4.42	3.92	4.01	3.81
<i>Caldimicrobium thiodismutans</i>	100.00	6.90%	3.98	4.19	3.61	1.95	2.46	4.17	3.59
<i>Thermus arciformis</i>	100.00	4.70%	3.47	1.67	3.38	3.89	4.03	3.67	3.63
<i>Thermocrinis ruber</i>	96.44	4.47%	2.65	2.59	3.39	4.00	4.25	1.67	2.40
<i>Fervidobacterium riparium</i>	100.00	2.92%	2.97	2.07	3.87	3.36	0.00	3.49	3.82
<i>Fervidobacterium pennivorans</i>	100.00	2.68%	3.40	3.61	3.30	3.86	0.00	2.46	3.42
<i>Meiothermus luteus</i>	100.00	2.23%	1.68	0.00	2.52	3.79	3.96	0.00	1.91
<i>Dictyoglomus turgidum</i>	99.21	2.16%	3.34	3.49	2.40	1.93	1.16	3.84	3.42
<i>Hydrogenobacter subterraneus</i>	96.44	2.07%	2.41	0.00	3.22	3.89	3.67	2.00	2.14
<i>Thermotoga caldifontis</i>	100.00	1.72%	3.45	3.50	2.97	1.43	0.00	2.57	3.68
<i>Thermus kawarayensis</i>	99.60	1.56%	3.40	2.19	3.01	3.38	3.42	3.01	3.05
<i>Thermodesulfovibrio yellowstonii</i>	98.02	1.12%	3.31	3.68	2.77	0.00	0.00	2.51	1.53

Excellent **abundance correlation** between *Chloroflexus* and *Caldimicrobium* in 7 diverse communities

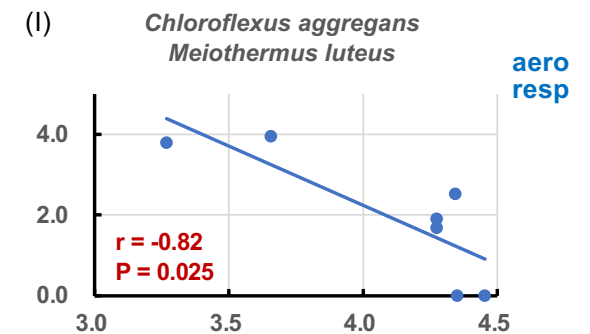
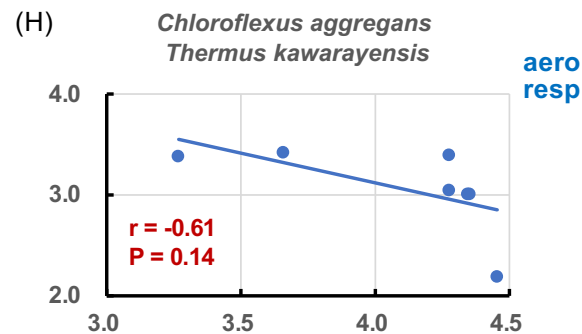
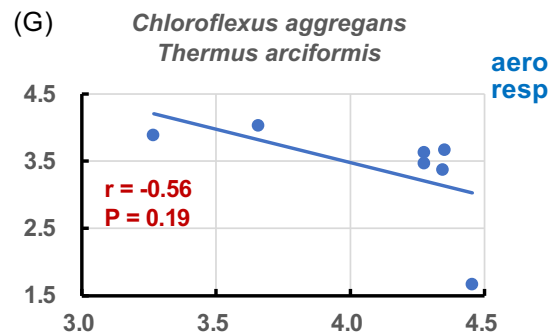
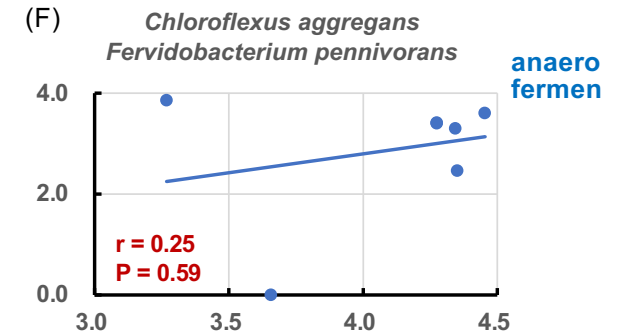
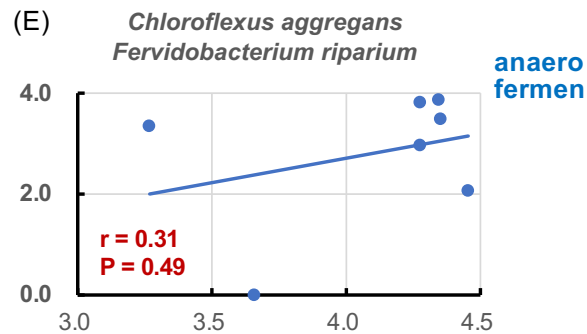
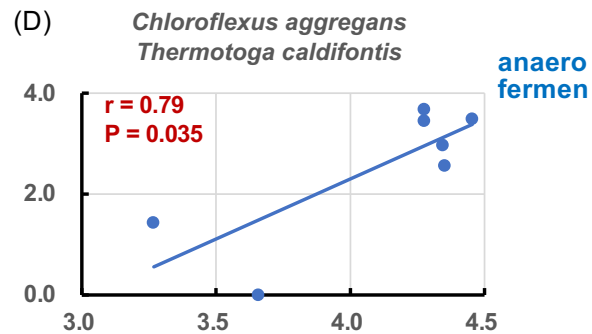
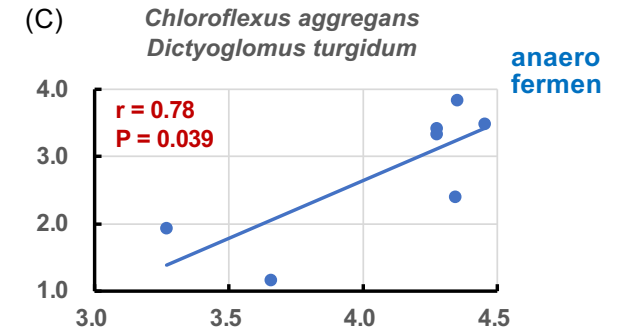
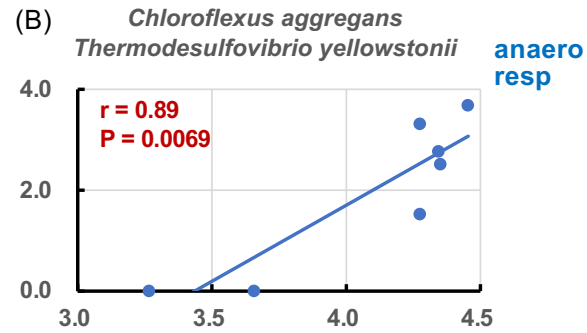
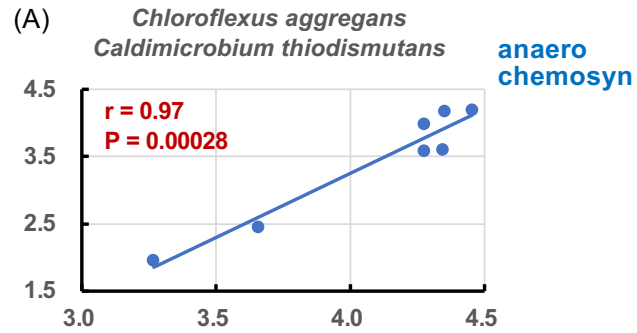


Abundance correlation coefficient (r) among 13 major known microbes

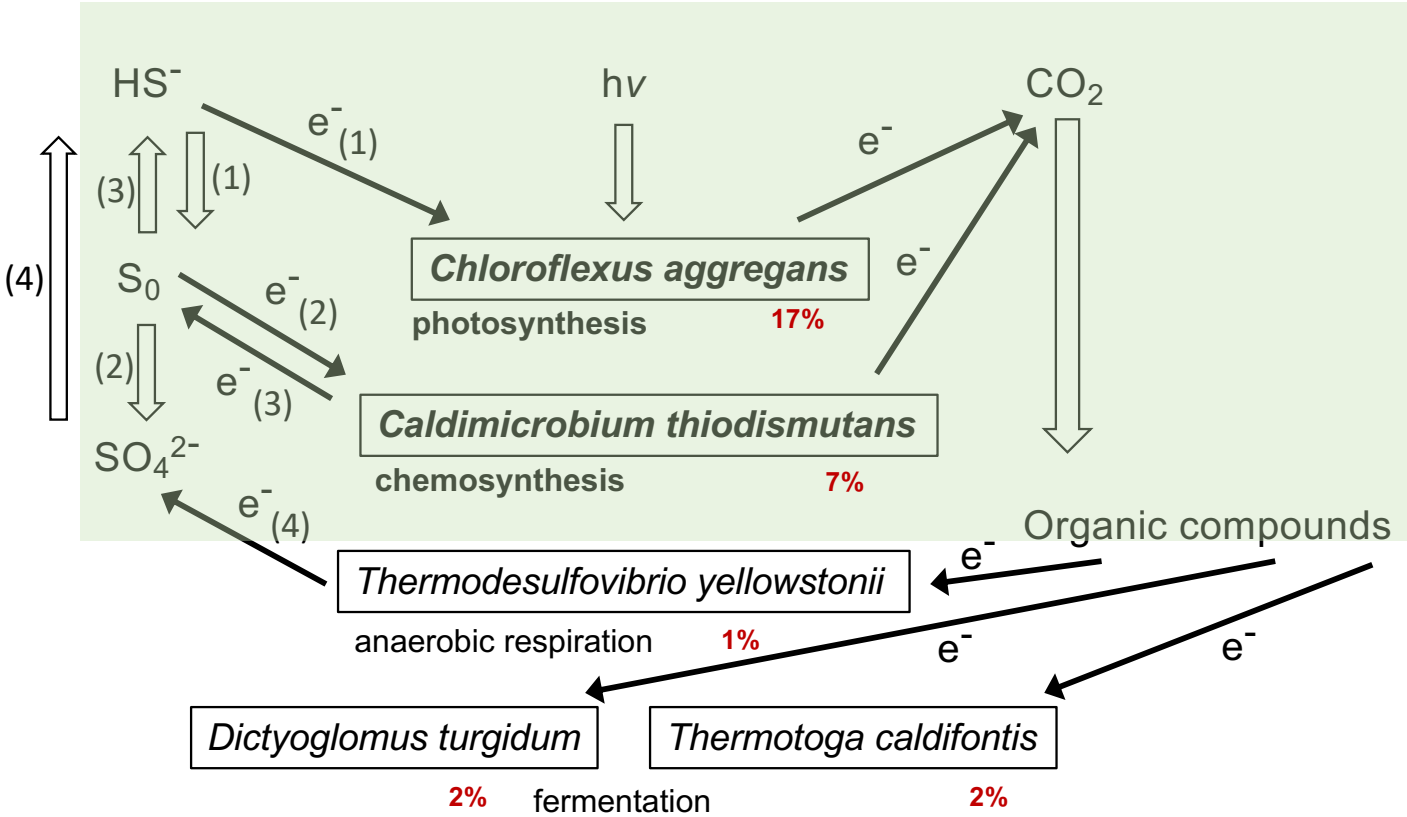
P value
 <0.0005
 <0.005
 <0.05

	C.a.	S.a.	C.t.	T.a.	T.r.	F.r.	F.p.	M.l.	D.t.	H.s.	T.c.	T.k.	T.y.
<i>Chloroflexus aggregans</i>		-0.66	0.97	-0.56	-0.78	0.31	0.25	-0.82	0.78	-0.73	0.79	-0.61	0.89
<i>Sulfurihydrogenibium azorense</i>	-0.66		-0.61	0.93	0.29	0.18	-0.14	0.59	-0.37	0.83	-0.47	0.90	-0.67
<i>Caldimicrobium thiodismutans</i>	0.97	-0.61		-0.57	-0.86	0.32	0.29	-0.91	0.87	-0.78	0.79	-0.60	0.93
<i>Thermus arciformis</i>	-0.56	0.93	-0.57		0.33	0.03	-0.41	0.65	-0.47	0.88	-0.54	0.93	-0.71
<i>Thermocrinis ruber</i>	-0.78	0.29	-0.86	0.33		-0.50	-0.37	0.91	-0.97	0.69	-0.74	0.45	-0.69
<i>Fervidobacterium riparium</i>	0.31	0.18	0.32	0.03	-0.50		0.80	-0.30	0.57	-0.06	0.68	-0.07	0.34
<i>Fervidobacterium pennivorans</i>	0.25	-0.14	0.29	-0.41	-0.37	0.80		-0.35	0.55	-0.34	0.76	-0.35	0.46
<i>Meiothermus luteus</i>	-0.82	0.59	-0.91	0.65	0.91	-0.30	-0.35		-0.91	0.88	-0.70	0.72	-0.82
<i>Dictyoglomus turgidum</i>	0.78	-0.37	0.87	-0.47	-0.97	0.57	0.55	-0.91		-0.76	0.86	-0.52	0.76
<i>Hydrogenobacter subterraneus</i>	-0.73	0.83	-0.78	0.88	0.69	-0.06	-0.34	0.88	-0.76		-0.67	0.88	-0.75
<i>Thermotoga caldifontis</i>	0.79	-0.47	0.79	-0.54	-0.74	0.68	0.76	-0.70	0.86	-0.67		-0.53	0.81
<i>Thermus kawarayensis</i>	-0.61	0.90	-0.60	0.93	0.45	-0.07	-0.35	0.72	-0.52	0.88	-0.53		-0.63
<i>Thermodesulfovibrio yellowstonii</i>	0.89	-0.67	0.93	-0.71	-0.69	0.34	0.46	-0.82	0.76	-0.75	0.81	-0.63	

Diverse abundance correlations with *C. aggregans*



Trophic interaction with *C. aggregans* in Nakabusa hot springs

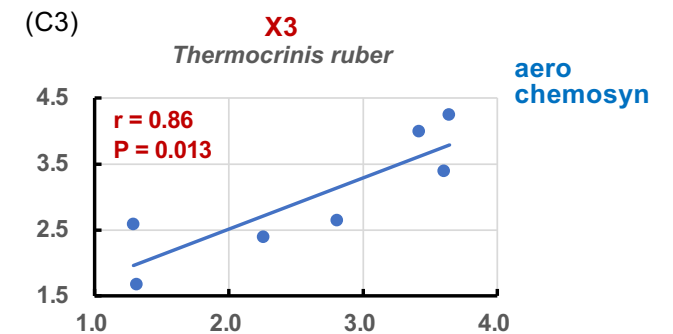
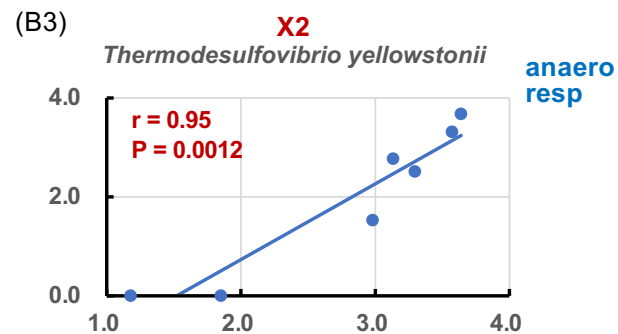
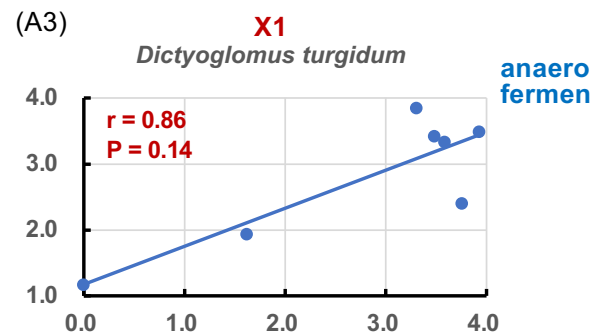
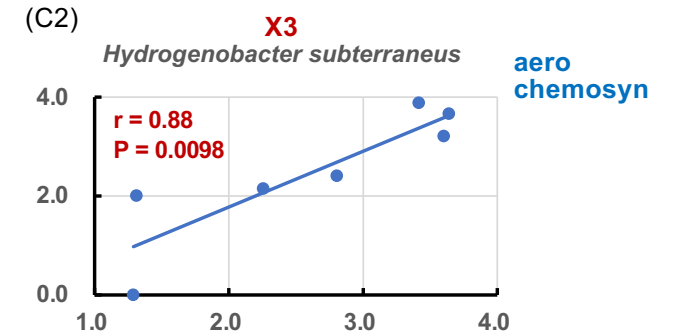
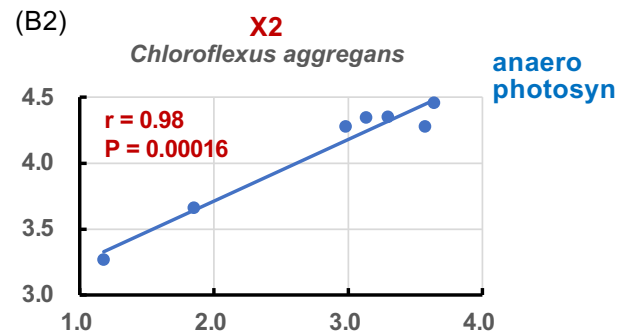
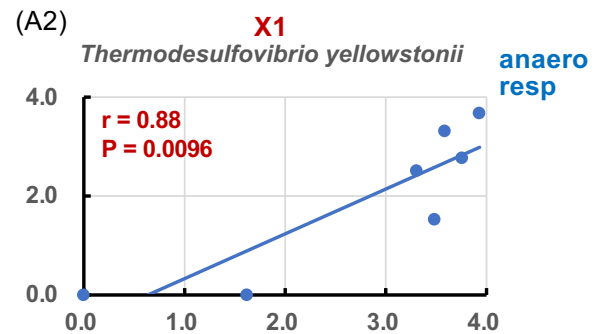
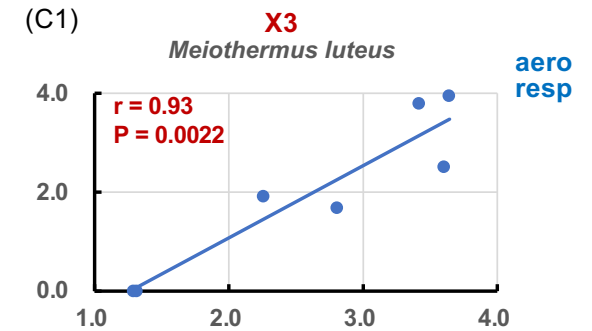
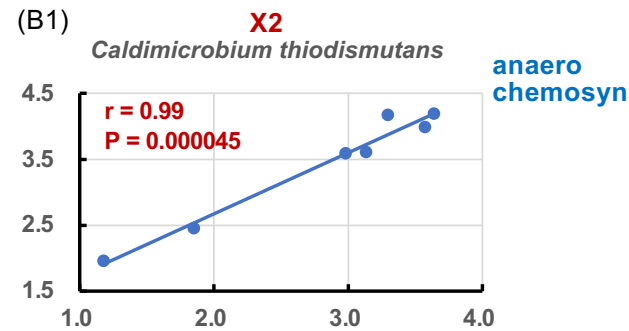
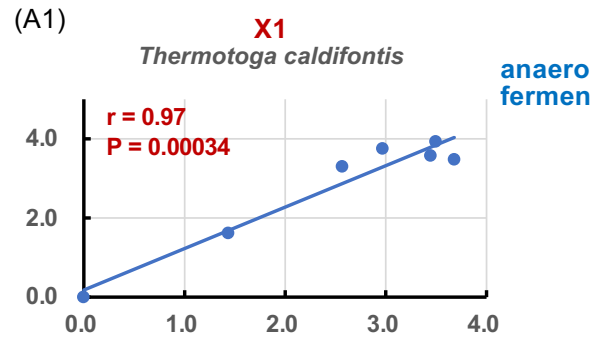


Speculation on **yet to be discovered microbes** from interactions

Relative abundance of three uncharacterized microbes (**X1, X2, X3**) in 7 diverse environments

	B1	B2	B3	B4	B5	B6	B7	nearest sp.	identity	ratio
X1	3.59	3.93	3.76	1.62	0.00	3.31	3.48	<i>Rhodothermus profundus</i>	87.14	3.32%
X2	3.57	3.64	3.14	1.18	1.85	3.30	2.98	<i>Thermobaculum terrenum</i>	86.17	1.79%
X3	2.81	1.29	3.60	3.42	3.64	1.31	2.26	<i>Carboxydothemus islandicus</i>	86.80	1.70%

Speculation on lifestyle of **yet to be discovered microbes** from correlation

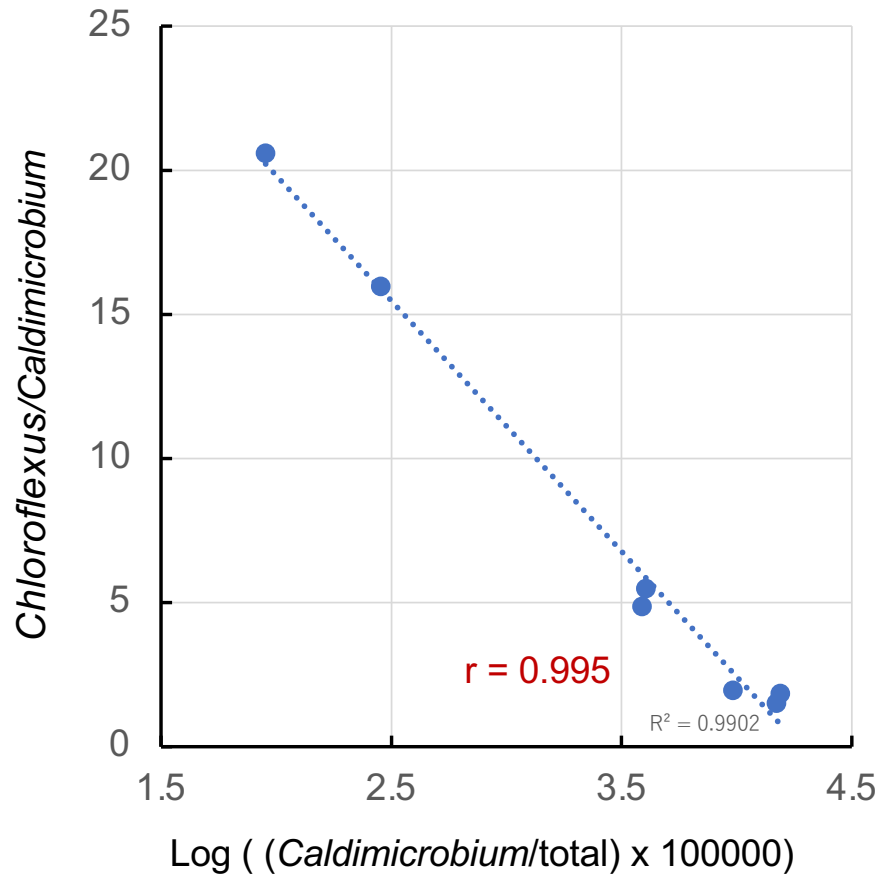


Conclusion

1. A **syntrophic relationship** between *C. aggregans* and *C. thiodismutans* **exists in natural communities** in Nakabusa hot springs.
2. *C. aggregans* were **also correlated with** the sulfate-reducing bacterium, *Thermodesulfovibrio yellowstonii*, and fermentative bacteria, *Dictyoglomus turgidum* and *Thermotoga caldifontis*.
3. Possible lifestyles of **uncultured microbes** were estimated from the **abundance correlation analysis** with co-existing known microbes.

Changes in the ratio of *C. aggregans* and *C. thiodismutans* in situ and in vitro

In natural communities at Nakabusa



In vitro co-culture

